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Hypergravity effects on succinate dehydrogenase reactivity in fish vestibular ganglia

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Abstract

Larval cichlid fish (*Oreochromis mossambicus*) were kept at 3*g* hypergravity (centrifuge) for 14 or 21 days. Subsequently, succinate dehydrogenase reactivity was histochemically demonstrated and densitometrically determined in the gangliones utricularis and saccularis as well as (for control) in the diencephalic, non-vestibular Nucleus glomerulosus posterioris.

It was found that succinate dehydrogenase reactivity within the ganglion utricularis was significantly increased in experimental animals as compared to the 1*g* controls ($p < 0.05$ and $p < 0.01$ after 14 and 21 days of hypergravity, respectively), whereas hypergravity had no effect on succinate dehydrogenase reactivity in the ganglion saccularis and in the Nucleus glomerulosus posterioris.

This result clearly indicates that hypergravity exclusively affects the metabolic activity of a ganglion, which is directly involved in the transmission of gravity inputs.

Keywords: Utricle; Saccule; Inner ear; Gravity

1. Introduction

Especially in developing vertebrates, altered gravitational environments such as weightlessness (microgravity, μ*g*) or hypergravity (h*g*, centrifuge) affect the physiology of cardiovascular, respiratory, intestinal, endocrine, immune, and muscular systems as well as the calcification of the skeleton and the neuronal computation of gravitational input (for review, see Seibert, 2001).

Concerning neuronal and vestibular plasticity, it was found using developing fish as model systems that a long-term adaptation to altered gravity is reflected in various compensation processes. On the level of the inner ear, hypergravity decreases inner ear otolith growth (Anken et al., 1998) as well as affects the reactivity of macular carbonic anhydrase (Beier et al., 2002), which is responsible for generating a pH-value necessary for otolith mineralization. Neuronal structures in developing fish are affected by altered gravity as well, particularly concerning synaptic plasticity (microgravity increases the number of synapses in a vestibular brain nucleus as if compensating the loss of vestibular input: Ibsch et al., 2000) or regarding brain energy and plasma metabolism. Whole-brain-biochemistry (for review, see Rahmann et al., 1995) showed that the activity of creatine kinase (CK) was augmented under simulated microgravity (clinostat), while glucose-6-phosphate dehydrogenase (G6PDH) activity was decreased. In comparison, a decrease in CK activity was found following h*g*-exposure, whereas the activities of G6PDH and succinate dehydrogenase (SDH, a marker enzyme of metabolic and thus neuronal activity) were reported to increase. Comparable, dose-dependent effects of altered gravity on SDH reactivity were found on histochemical level in individual vestibular brain nuclei (increasing SDH reactivity from μ*g* via 1*g* to h*g*), whereas non-vestibular brain nuclei were not affected by the altered gravitational environment (Anken et al., 1994).

The vestibular ganglia, which comprise neurons directly connecting the inner ear maculae to the brain, have

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hitherto been neglected in studies on the effect of altered gravity on neuronal plasticity. We were thus prompted to densitometrically determine SDH reactivity in the ganglione utricularis and saccularis of larval cichlid fish, which had been maintained at hg during a 14 or 21 days period in comparison to 1*g* controls. A subunit of the diencephalic Nucleus glomerulosus posterioris was exemplarily analyzed as a non-vestibulum related area within the brain.

2. Material and methods

Larval cichlid fish siblings (*Oreochromis mossambicus*, Perciformes) of stage 12 (approximately 6 days after fertilization at 20 °C, prominent yolk sac; Anken et al., 1993) were kept at 22 °C in the dark either at 1*g* normal earth gravity (1*g* controls) or at 3*g* hypergravity (hg, centrifuge). Details on the centrifuge were published elsewhere (Ibsch et al., 1999). This "Video Observation Centrifuge for Aquatic Vertebrates, VOCAV" is equipped with a (rotating) camera allowing to observe the behaviour of the animals during hg. For observation, illumination was switched on for some 2 min per day.

After 14 and again after 21 days, the centrifuge was stopped, larvae were removed, sacrificed in ice water and cryoprotected by a solution of 20% sucrose in 0.1 M phosphate buffer, pH 7.4, for 40 min at 22 °C, followed by an incubation in two parts 20% sucrose : 1 part OCT (Tissue Tec Compound Medium, Ted Pella, USA) for 60 min at 22 °C. After cryoprotection, the samples were frozen at −80 °C within the medium containing two parts OCT : 1 part 20% sucrose in isopentane.

SDH reactivity was demonstrated on 10 μm thin cryostat transverse sections using an incubation medium of 0.1 M phosphate buffer, pH 7.4, (PB) containing 1 mg/ml nitro blue tetrazolium chloride and 6.8 mg/ml succinic acid (dipotassium-salt-hexahydrate). Incubation was carried out at 37 °C and lasted 90 min. After incubation, sections were rinsed in PB and in bidistilled water, briefly dehydrated in an ascending series of ethanol and mounted in Hydromatrix (Micro-Tech-Lab, Austria). All sections which were later to be compared with each other concerning SDH reactivity in neuronal structures were always handled simultaneously.

Densitometric readings of SDH reaction product within the central neuropil of the diencephalic Nucleus glomerulosus posterioris (Figs. 1a and a′; $n = 4$ hg-animals and $n = 4$ 1*g* controls each after 14 and 21 days) as well as within the areas covered by the ganglion utricularis ($n = 6$ hg/$n = 6$ 1*g*) and the ganglion saccularis ($n = 3$ hg/$n = 3$ 1*g*) (Figs. 1b, b′) were carried out on all sections showing the respective structures using a computer-based image analysis system (Axio Cam MRC, Axiovision 3.06, Zeiss, Germany). Measuring was done at 540 nm, which corresponds to the absorbance maximum of the final reaction product.

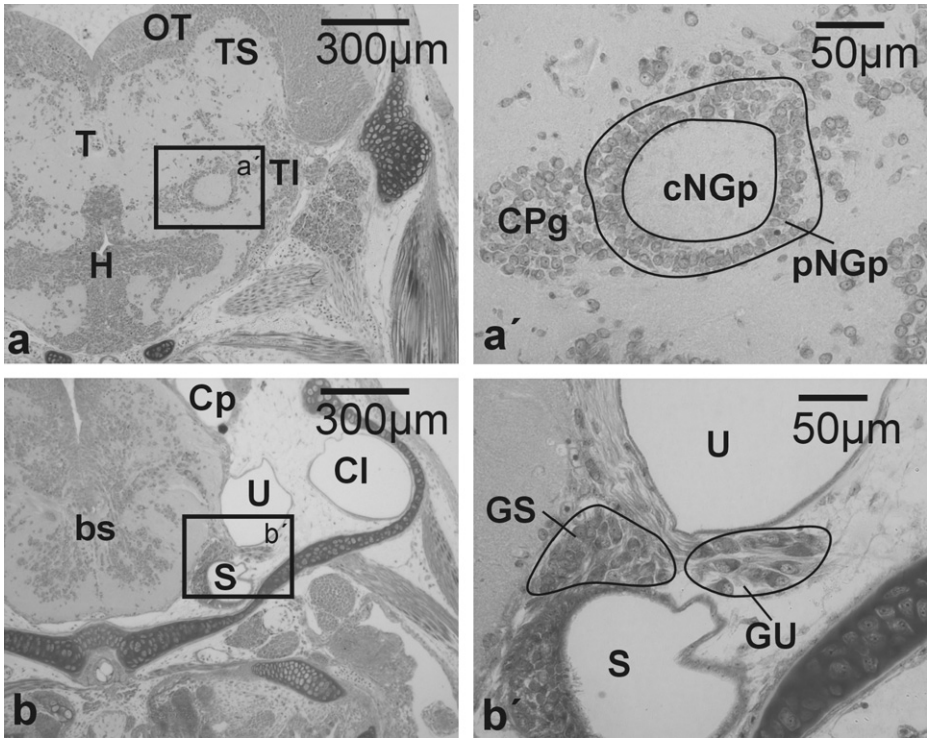

Fig. 1. Cytoarchitectonic localization of the neuronal structures examined. (a and b) Low magnification partial microphotographs (right side of head) of transverse resin sections, thickness 3 μm, toluidine blue staining. The rectangles refer to detailed images in (a′ and b′). (a and a′) Nucleus glomerulosus posterioris, NGp. cNGp, central neuropil of NGp; CPg, preglomerular complex; H, hypothalamus; OT, optic tectum; pNGp, peripheral neurons of NGp; T, thalamus; Tl, torus lateralis; TS, torus semicircularis. (b and b′) Ganglion utricularis, GU, and ganglion saccularis, GS. bs, brain stem; Cl, canalis lateralis; Cp, canalis posterioris; S, saccule; and U, utricle.